EVIDENCE FOR THE EXISTENCE OF A FRACTION OF RAT LIVER CHROMATIN CONTAINING ONLY A FEW SPECIFIC NONHISTONE PROTEINS AND REDUCED AMOUNT OF HISTONE H1

Boika ANACHKOVA and George RUSSEV*
Institute of Biochemistry, Bulgarian Academy of Sciences, 1113-Sofia, Bulgaria

Received 15 June 1977

1. Introduction

In previous papers we have shown [1,2] that upon alkaline treatment about 70% of the nonhistone chromosomal (NHC) proteins together with most of the histones were dissociated from rat liver chromatin. Only a few NHC-proteins remained complexed to DNA, the major two of which were identified as α- and β-tubulin [3–5]. It was concluded that these proteins were most probably directly held to DNA and might play some specific role in chromatin architecture. In the present paper we provide evidence that there are discrete fractions of rat liver chromatin containing exclusively the same NHC-proteins. These fractions contain no, or only very little, histone H1 and could serve as specific 'adhesive sites' along the nucleoprotein threads.

2. Materials and methods

Rat liver chromatin was isolated by extraction of Nonidet P40 nuclei with solutions of increasing NaCl concentrations up to 0.35 M as previously described [6]. After swelling overnight in deionized water the chromatin was dissolved by homogenization in deionized water (10–15 A_{260}/ml) and was extensively fragmented by sonication with an MSE ultrasonic unit in 10 ml portions at 1 A for 5 × 1 min, in an ice bath. The chromatin solution was then clarified by centrifugation at 20 000 rev/min in a Beckman J-21B centrifuge at 4°C for 40 min and the clear supernatant was made 0.5 mM in GTP and 0.1 mM in Mg^{2+} by adding 1/10 vol 5.5 mM GTP (Koch-Light), 1.1 mM $MgCl_2$ in 0.2 M ACES (BDH), pH 6.5. The mixture was incubated at 37°C for 40 min and was then centrifuged again at 20 000 rev/min for 40 min, this time at 30°C. The pellet was suspended by sonication (5 × 1 min in an ice bath) in 10 mM EDTA, pH 8, to make a solution of about 20 A_{260}/ml. The solution was clarified by centrifugation at 5000 rev/min for 10 min and the chromatin was precipitated from the supernatant with 1/10 vol. 1.5 M NaCl.

DNA was isolated by dissolving the chromatin in 1% SDS, 1 M NaCl, 0.02 M EDTA, 0.05 M Tris–HCl, pH 7.5 and digesting the proteins with 200 μg/ml of proteinase K (Merck) at 60°C for 2 h. The samples were deproteinized by phenol–chloroform and chloroform extractions and finally purified on a hydroxyapatite column.

SDS–polyacrylamide-gel electrophoresis was carried out after Laemmli [7] as previously described [2].

Protein was determined by the method of Lowry et al. [8], and DNA by the method of Burton [9].

3. Results and discussion

On incubation at 37°C in the presence of Mg^{2+} and GTP, tubulin forms microtubules which can be collected by centrifugation at 30 000–50 000 × g [10]. We used this property of tubulin to check its recently reported presence in rat liver chromatin [2,11]. Rat liver chromatin with a histone : NHC-protein : DNA

*To whom correspondence should be addressed

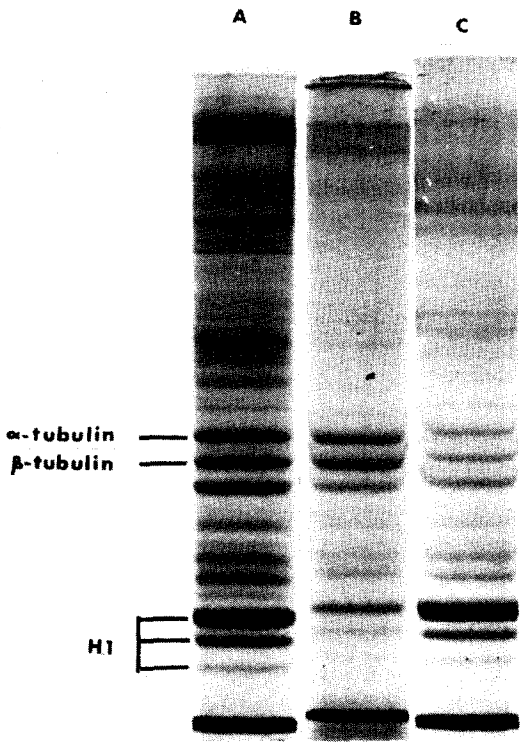

Fig.1. SDS–polyacrylamide-gel electrophoresis (8.75% gel) of total rat liver chromatin (A), the sediment obtained after incubation of the sonicated chromatin with GTP and Mg^{2+} (B), and the corresponding supernatant (C).

ratio of 1:1.2:1 was sonicated to give fragments of about 500–600 base pairs as evidenced by agarose–gel electrophoresis of the corresponding DNA (not shown here). On centrifugation at 20 000 rev/min at 4°C for 40 min about 10% of the starting material sedimented and the remaining 90% were recovered in the clear supernatant. SDS–polyacrylamide-gel electrophoresis revealed no differences in the protein complement of this first sediment and the supernatant. The supernatant was made 0.5 mM in GTP and 0.1 mM in Mg^{2+}, pH 6.5 (to keep ionic strength low, ACES was used) and was incubated at 37°C for 40 min. A faint turbidity developed in the course of incubation which could be followed by measuring the absorption at 340 nm. The precipitated material was pelleted by centrifugation. To make sure that some of the tubulin dissociated during sonication had not coprecipitated with this chromatin fraction, the pellet was suspended under conditions known to completely destroy microtubules and the nucleoprotein was then precipitated in, and washed with, 0.15 M NaCl. In this way 3–4% of the starting chromatin were recovered. It showed a histone : NHC-protein : DNA ratio of 0.6–0.8:1–1.2:1. SDS–polyacrylamide-gel electrophoresis revealed that this fraction of rat liver chromatin contained only a few NHC-proteins the major two of which are α- and β-tubulin [3–5]. Several high molecular weight fractions and the fraction comigrating with actin [3] were also present, while the amount of the remaining NHC-proteins and of histone H1 was strongly reduced. As evidenced by the kinetics of reassociation followed on a hydroxyapatite column for several Cot-values in the range 0.2–20, about 30% of the DNA of the pellet consisted of repeated sequences, this percentage being the same as that of the total rat genome.

We concluded that there are fractions of rat liver chromatin where DNA is predominantly complexed to tubulin or to some tubulin-like proteins capable of self-organization. These fractions represent sorts of adhesive sites along chromatin fibres and may play some role in the process of chromatin condensation.

References

[1] Russev, G., Venkov, C. and Tsanev, R. (1974) Eur. J. Biochem. 43, 253–256.
[2] Russev, G., Anachkova, B. and Tsanev, R. (1975) Eur. J. Biochem. 58, 253–257.
[3] Anachkova, B., Russev, G. and Tsanev, R. (1977) Int. J. Biochem. 8, 135–139.
[4] Anachkova, B., Russev, G. and Tsanev, R. (1977) Int. J. Biochem. in press.
[5] Gineitis, A., Anachkova, B. and Russev, G. (1977) submitted.
[6] Tsanev, R. and Russev, G. (1974) Eur. J. Biochem. 43, 257–263.
[7] Laemmli, U. K. (1970) Nature 227, 680–685.
[8] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[9] Burton, K. G. (1956) Biochem. J. 62, 315–323.
[10] Borisy, G. G. and Olmsted, J. B. (1972) Science 177, 1196–1197.
[11] Douvas, A. S., Harrington, C. A. and Bonner, J. (1975) Proc. Natl. Acad. Sci. USA 72, 3902–3906.